

Validation data for HEK-Blue™ IL-10 cells

<https://www.invivogen.com/hek-blue-il10>

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Version 19K25-NJ

HEK-Blue™ IL-10 cells allow the detection of bioactive human interleukin-10 (hIL-10) by monitoring STAT3 activation. They were generated by stable transfection of HEK293 cells with the genes encoding the IL-10R receptor, STAT3, and a STAT3-inducible SEAP (secreted embryonic alkaline phosphatase) reporter. These cells display high sensitivity to hIL-10 (detection range: 0.3-30 ng/ml; **figure 1**) and low sensitivity to mIL-10 (detection range: 25-1000 ng/ml; **figure 2**, and not shown). Because STAT3 is also implicated in the signaling of cytokines that do not belong to the IL-10 family, such as IFN- α/β and IL-6, HEK-Blue™ IL-10 cells are knockout for the expression of hIFNAR2 and hIL-6R. HEK-Blue™ IL-10 cells are highly specific for the detection of human and murine IL-10, and are non-responsive to hIL-1 β , hIL-2, hIL-6, hIL-12, hIL-22, hIL-23, hIFN- α , hIFN- β , hIFN- γ , and hTNF- α (**figure 2**). HEK-Blue™ IL-10 cells can be used for screening antibodies targeting the IL-10/IL-10R pathway (**figure 3**).

Cellular response to hIL-10

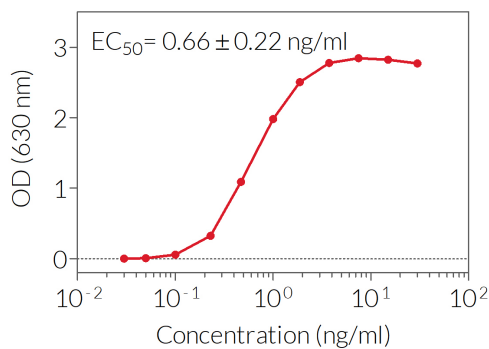


Figure 1. Dose-response of HEK-Blue™ IL-10 cells to recombinant hIL-10. Cells were stimulated with increasing concentrations of recombinant human IL-10. After overnight incubation, the STAT3 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and reading the optical density (OD) at 630 nm. EC₅₀ value is shown as mean \pm SD.

Cell line specificity

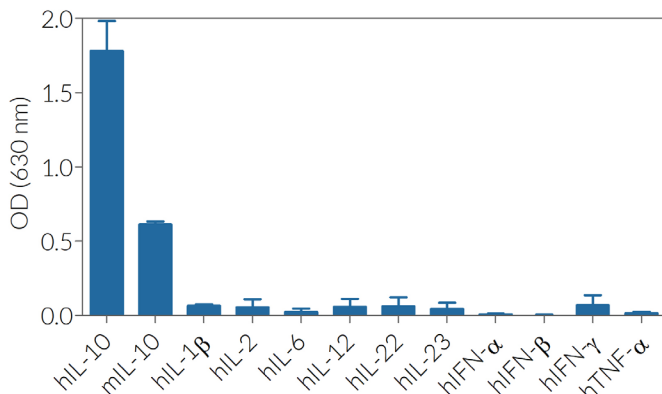


Figure 2. Response of HEK-Blue™ IL-10 cells to a panel of cytokines. Cells were stimulated with various human and murine recombinant cytokines: 1 ng/ml of hIL-10, 30 ng/ml of mIL-10, 1 ng/ml of hIL-1 β , hIL-2, hIL-6, hIL-12, hIL-22, hIL-23, hIFN- γ , hTNF- α , or 10² U/ml hIFN- α or - β . After overnight incubation, SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) at 630 nm is shown as mean \pm SEM.

Human IL-10 signaling inhibition

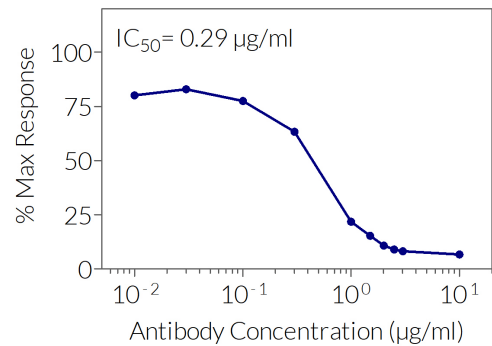


Figure 3. Dose-dependent inhibition of HEK-Blue™ IL-10 cellular response using a neutralizing antibody against the hIL-10 receptor (hIL10R). The anti-hIL10R antibody was incubated with the cells for 30 minutes prior to the addition of hIL-10 (0.5 ng/ml). After overnight incubation, SEAP activity in the cell culture supernatant was assessed using QUANTI-Blue™ Solution. Data represent % of maximal reporter activity without the anti-hIL10R antibody.

TECHNICAL SUPPORT

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